Dynamic Adaptive Response of Pseudomonas Aeruginosa to Clindamycin/Rifampicin-Impregnated Catheters

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ABSTRACT

Pseudomonas aeruginosa is the most common Gram-negative pathogen causing nosocomial pneumonia; it is often multidrug-resistant, a good biofilm-producer and has the potential for contaminating antimicrobial-impregnated medical devices. Despite the widespread use of antimicrobial-impregnated catheters, little is known about their effects on antibiotics in P. aeruginosa. In this study, we investigated the adaptive resistance potential of P. aeruginosa strain PAO1 in response to continuous antibiotic exposure from clindamycin/rifampicin-impregnated catheters (CR-IC). During exposure for 144 hr to clindamycin and rifampicin released from CR-IC, strain PAO1 formed biofilms featuring elongated and swollen cells. There were 545 and 372 differentially expressed proteins identified in the planktonic and biofilm cells, respectively, by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Both Cluster of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses showed that the planktonic cells responded to the released antibiotics more actively than the biofilm cells, with metabolism and ribosomal biosynthesis-associated proteins being significantly over-expressed. Not only were certain groups of virulence proteins, including the outer membrane-associated (flagella, type IV pili, type III secretion system) and extracellular (pyoverdine) virulence proteins, up-regulated, but also the phenotypic invasion capability for HeLa cells was increased. Continuous exposure of P. aeruginosa to CR-IC induced over-expression of antibiotic resistance proteins, including porin, efflux pump, translation and transcription proteins even though the upregulation did not affect the change of phenotypic minimum inhibitory concentration (MIC) during the experimental timeframe. The concerning association between CR-IC and virulence factors suggests the need for additional investigation to determine if it results in adverse clinical outcomes.

INTRODUCTION

More than 30 million urinary tract catheters, five million central venous catheters, two million fracture fixation devices, and one million dental implants are used in the US annually. Although medical devices are widely used for therapeutic treatments, infections associated with the presence of a device result in significant morbidity and mortality for patients. Nosocomial bacteria can contaminate the surgical site surrounding implants and adhere to their surface and form biofilm, resulting in evasion of the host immune response and strong resistance to many antimicrobials. If antimicrobial therapy is discontinued, persister cells can detach from the biofilm and cause recurrent infection. Host factors, such as fibronectin and fibrin, and material surface characteristics, such as roughness and hydrophobicity, influence biofilm formation. Biofilm can also prevent devices from functioning properly. Biofilms formed on intravascular catheters or central lines are recognized to be the most common cause of hospital-acquired bloodstream infections. Catheter-related bloodstream infection rates were 5.0 and 8.5 per 1,000 central-line days for surgical intensive care and burn units each year, respectively. In order to control biofilm colonization, the internal and external surfaces of venous catheters may be coated with antibiotics. Two commercially available catheters impregnated with combinations of clindamycin/rifampicin and minocycline/rifampicin have been approved by the US Food and Drug Administration (FDA). Previous reports that clindamycin/rifampicin- and minocycline/rifampicin-coated catheters had no effect on the growth of P. aeruginosa have increased concern over the possible development of microbial resistance to the antibiotics employed in the catheters. Therefore, it is important to understand how P. aeruginosa may overcome these antibiotic-impregnated catheters, and whether the presence of the antimicrobial in devices can lead to antimicrobial resistance. To the best of our knowledge, no studies have been conducted to investigate the potential of adaptive resistance and cross-resistance to antibiotics after exposure of P. aeruginosa to the antibiotic-coated catheters. In this study, we investigated the response of P. aeruginosa PAO1 to the antibiotics eluted from clindamycin/rifampicin-impregnated catheters (CR-IC), using genetic, minimum inhibitory concentration (MIC), and proteomic analyses, to evaluate the potential development of adaptive and cross-resistance.

RESULTS

Bacteria growth test in antimicrobial-coated catheters

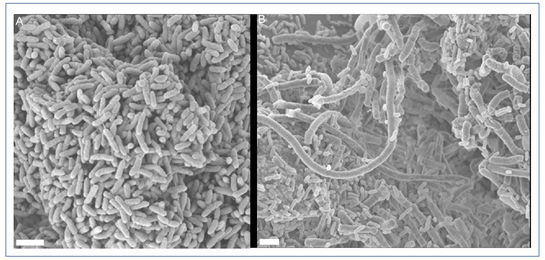
Catheters	24 h		48 h		72 h	
	PAO1	TCH1516	PAO1	TCH1516	PAO1	TCH1516
BHI only	+	+	+	+	+	+
Control catheter	+	+	+	+	+	+
Clindamycin/rifampin	+	-	+	-	+	-
Minocycline/rifampin	+	-	+	-	+	-

Overview of proteome data

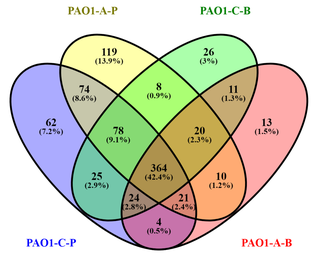
	PAO1-C-P	PAO1-A-P	PAO1-C-B	PAO1-A-B
Total protein numbers	694	652	556	467

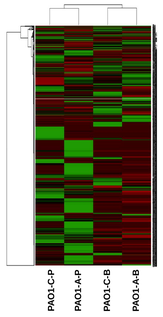
PAO1-A-P/PAO1-C-P		PAO1-A-B/PAO1-C-B	
Up	Down	Up	Down
326	219	137	235

FESEM images of *P. aeruginosa* PAO1 biofilm cells

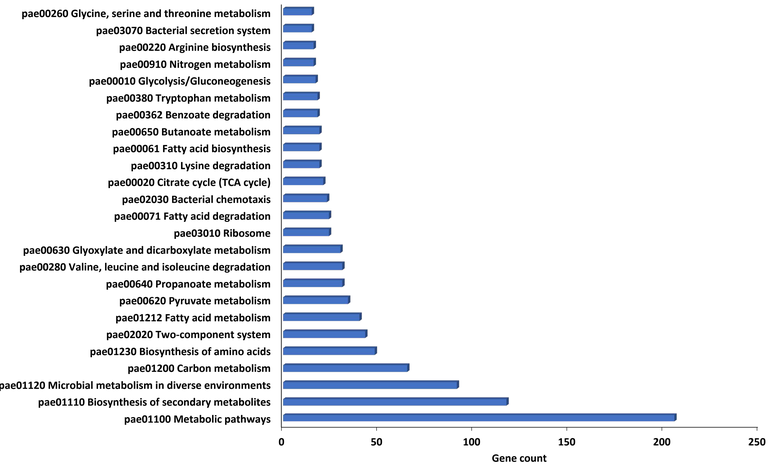


Venn diagram and heatmap analysis of proteomic data





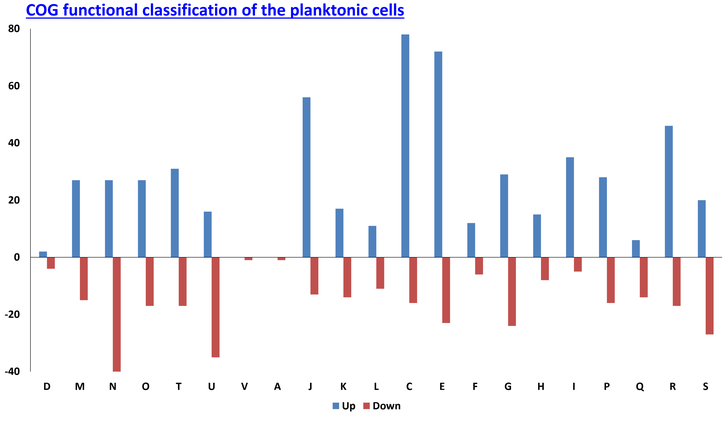
Top 25 KEGG pathways of up-regulated proteins from the planktonic cells



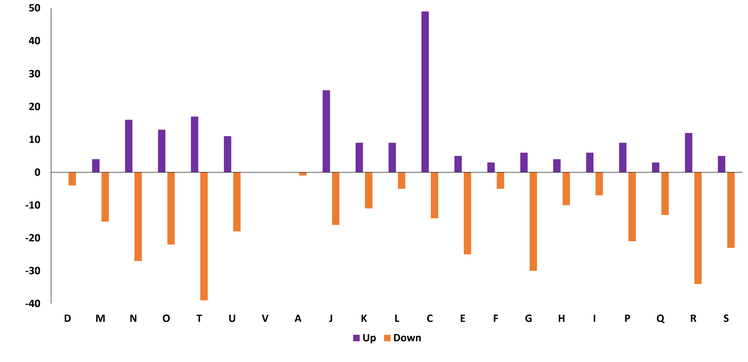
Identified proteins associated with antibiotic resistance of planktonic cells

Locus Tag	Gene	Product	Fold Ratio
PA0156	triA	Multidrug efflux system, membrane fusion component	0.97
PA0291	oprE	Outer membrane low permeability porin, OprD family	0.00
PA0425	mexA	Multidrug efflux system, membrane fusion component	0.71
PA0427	oprM	Multidrug efflux system, outer membrane factor lipoprotein	18.38
PA0958	oprD	Outer membrane low permeability porin, OprD family	13.16
PA1178	oprH	PhoP/Q and low Mg2+ inducible outer membrane protein H1 precursor	1000.00
PA1777	oprF	Nonspecific porin and structural outer membrane protein OprF	1.41
PA2071	fusA2	Translation elongation factor G	1.37
PA2291	oprB2	probable glucose-sensitive porin	1000.00
PA2398	fpvA	ferripyoverdine receptor	1000.00
PA2525	opmE	Outer membrane factor (OMF) lipoprotein associated wth MdtABC efflux system	0.00
PA2950	fabV	Enoyl-[acyl-carrier-protein] reductase [NADH]	6.63
PA3186	oprB	Glucose/carbohydrate outer membrane porin OprB precursor	1000.00
PA4248	rplF	LSU ribosomal protein L6p (L9e)	3.21
PA4264	rpsJ	SSU ribosomal protein S10p (S20e)	19.09
PA4266	fusA1	Translation elongation factor G	3.45
PA4269	rpoC	DNA-directed RNA polymerase beta' subunit	6.81
PA4270	rpoB	DNA-directed RNA polymerase beta subunit	13.15
PA4277	tufB	Translation elongation factor Tu	3.46
PA4974		Outer membrane channel TolC (OpmH)	1.04
PA5239	rho	Transcription termination factor Rho	6.61

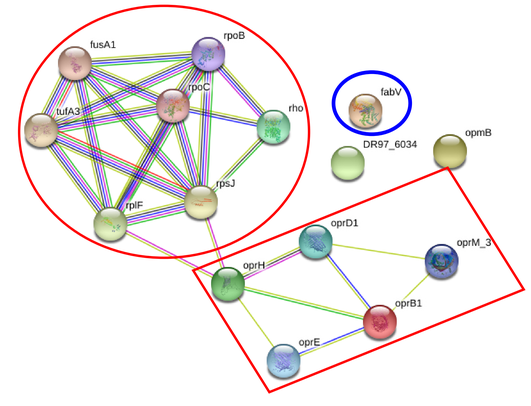
COG functional classification of the planktonic cells



COG functional classification of the biofilm cells



STRING protein-protein interaction network analysis of differentially expressed proteins associated with antibiotic resistance of planktonic cells



SUMMARY

- During continuous culture with the antibiotic-coated catheters, strain PAO1 survived and formed biofilms on catheters, featuring elongated and swollen cells
- From the proteomic data analysis, 694 and 556 proteins were identified from the planktonic and biofilm cells in control catheters, and 652 and 467 proteins from the planktonic and biofilm cells in antibiotic-coated catheters
- Both COG and KEGG analyses showed that the planktonic cells responded to the released antibiotics more actively than the biofilm cells, with metabolism and ribosomal production-associated proteins being significantly over-expressed
- Continuous exposure of the antibiotic-coated catheters to PAO1 induced over-expression of antibiotic resistance proteins, including porin, efflux pump, translation and transcription proteins
- Not only were certain groups of virulence proteins, but also the phenotypic invasion capability was increased